A5 CONT

medium), the calculated biliary clearance value indicating the susceptibility of the metabolite of the candidate parent compound to biliary excretion.

Please add the following new claims:

A6

65. (New) The method of claim 39, further comprising the step of differentiating between a candidate compound that is not excreted in bile, a candidate compound that is highly excreted in bile, and a candidate compound that is readily and extensively excreted in bile, using the biliary clearance value.

66. (New) The method of claim 52, further comprising differentiating between a candidate compound that is not excreted in bile, a candidate compound that is highly excreted in bile, and a candidate compound that is rapidly and extensively excreted in bile, using the biliary clearance value.

REMARKS

Status Summary

The United States Patent and Trademark Office (hereinafter the "Patent Office" has examined claims 1-64, as presented in the present U.S. patent application, which was filed on March 17, 2000. The Patent Office has issued a non-final first Official Action. As set forth in the Official Action, claims 1-64 are rejected. More specifically, claims 12, 24, 39, and 52 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

Claims 1-11, 13-23, and 25-38 are rejected under 35 U.S.C. § 102(b) as being anticipated by LeCluyse et al. [U] or Liu et al. [EE]. Claims 1-64 are rejected under 35 U.S.C. § 102(a) as being anticipated by Liu et al. [CC]. Claims 1-64 are rejected under 35 U.S.C. § 103(a) as being unpatentable over LeCluyse et al. [U] and/or Liu et al. [EE] in view of Dunn [A] (U.S. Patent No. 5,602,026), Liu et al. [DD], and Poole et al. [V].

Claims 12 and 24 have been cancelled. Claims 1, 13, 25, 39, and 52 have been amended. New claims 65 and 66 have been added. The amendments to the claims are intended to more particularly point out and distinctly claim the subject invention. No new matter has been added by virtue of any of the amendments to the claims.

Reconsideration of the present U.S. patent application as amended and based on the arguments set forth below is respectfully requested.

Pages Showing Changes Made

Pursuant to 37 CFR § 1.121, attached hereto is a marked-up version of the claims, which illustrates all of the changes made thereto. The attached page is captioned "Version With Markings To Show Changes Made". Deleted language is bracketed and added language is underlined.

Response to the Rejection of Claims Under 35 U.S.C. §112

The Patent Office has rejected claims 12, 24, 39, and 52 under 35 U.S.C. Section 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. More specifically, the Patent Office asserts that claims 12, 24, 39, and 52 are rendered indefinite by the phrase "calculating a biliary clearance value" upon the contention that this phrase fails to particularly point out and distinctly claim the subject matter of the invention. Applicant has cancelled claims 12 and 24, thus rendering this rejection moot with respect to these claims.

To more particularly point out and distinctly claim the subject matter of the subject invention, claims 39 and 52 have been amended to include the disclosure located at page 19, lines 19-24, of the specification. Applicant respectfully submits that the rejection under 35 U.S.C. Section 112, second paragraph, of claims 39 and 52 has been overcome. Withdrawal of this rejection and allowance of claims 39 and 52 are respectfully requested.

Response to the Rejection of Claims Under 35 U.S.C. Section 102(b)

Based on LeCluyse et al. [U] or Liu et al. [EE]

Claims 1-11, 13-23, and 25-38 stand rejected under 35 U.S.C. Section 102(b) as being anticipated by LeCluyse et al. [U] or Liu et al. [EE]. The Patent Office asserts that LeCluyse et al. [U] or Liu et al. [EE] disclose identical active steps and identical

structural elements of the subject invention as claimed. More specifically, the Patent Office asserts that LeCluyse et al. [U] disclose a method of screening candidate compounds, such as carboxyfluorescien and rhodaminephaloidin for susceptibility to biliary excretion, and that Liu et al. [EE] disclose a method of screening a plurality of candidate compounds, such as taurocholate, radiolabeled enkephalin, alanine, morphine, inulin, etc. for susceptibility to biliary excretion. This rejection is respectfully traversed.

Unlike the present invention, LeCluyse et al. [U] and Liu et al. [EE] do not teach, suggest, or motivate a method of providing an *in vitro* culture of hepatocytes having at least one bile canaliculus, which can be utilized for screening candidate compounds to indicate the susceptibility of a candidate compound to biliary excretion *in vivo.* By indicating the susceptibility of a candidate compound to biliary excretion *in vivo*, candidate compounds having an undesirably high susceptibility can optionally be eliminated early on from further evaluation as a potential therapeutic agent. Therefore, the subject invention enables early developmental drug screening to, in essence, "weed out" the therapeutic compounds that have only a minimal chance of imparting therapeutically beneficial effects upon a patient due to their high susceptibility to biliary excretion *in vivo*.

In marked contrast, LeCluyse et al. [U] are merely concerned with studying the effects on *in vitro* hepatocytes cultured in various collagen configurations in an effort to determine which collagen configuration would promote the formation of extensive *in vitro* bile canalicular networks so as to attempt to provide a more reliable representative model with which to study hepatic morphology and physiology. Given the abbreviated nature of this abstract, Liu et al [EE] merely discloses the measurement of *biliary excretion index* for selected model compounds in sandwich-cultured hepatocytes. Unlike the present invention, LeCluyse et al. [U] and Liu et al. [EE] do not teach, suggest, or motivate a method of screening compounds *in vitro* for determining the susceptibility of a compound to *in vivo* biliary excretion wherein an amount of candidate compound in the at least one bile canaliculi has been normalized. As a result, LeCluyse et al. [U] and Liu et al. [EE] suffer from a significant weakness in ability to predict *in vivo*

excretion based on *in vitro* observations. Claims 1 and 13 have been amended to recite this aspect of the present invention, and are thus believed to be patentably distinguished over LeCluyse et al. [U] and Liu et al. [EE]. Support for this amendment can be found throughout the subject application as filed, including at page 30, lines 19-24. Normalizing the amount of candidate compound facilitates accurate extrapolation and scaling up of the *in vitro* observations to an *in vivo* setting, in accordance with the present invention.

Claim 25 recites a competition assay in accordance with the present invention, wherein a candidate compound and a marker compound are competed against each other to provide a method of screening a candidate compound for susceptibility to biliary excretion that is amenable to a high throughput screening approach. Thus, both a candidate compound and a marker compound are added to the culture for a time sufficient to allow for uptake so that the candidate compound and marker compound compete for uptake in the culture of hepatocytes. This aspect of the invention is now believed to be more particularly pointed out and distinctly claimed through the amendment of claim 25, step (d), to recite that the detection of the marker compound is done to evaluate uptake and excretion competition between the candidate compound and the marker compound. Support for this amendment can be found in the subject U.S. Patent Application as filed at page 22, line 8 through page 24, line 22, and particularly at page 22, lines 20-21. The evaluating of uptake and excretion competition between the candidate compound and the marker compound is not disclosed in the Liu et al. [EE] nor the LeCluyse et al. [U] references in any manner whatsoever. Therefore, claim 25 and its dependent claims 26-38 are believed to be patentably distinguished over the cited art of record. Allowance of these claims is respectfully requested.

Because of the aforementioned discussion regarding the patentability of amended claims 1, 13, and 25, and because claims 2-11 are dependent upon claim 1, claims 14-23 are dependent upon claim 13, and claims 26-38 are dependent upon claim 25, applicants respectfully submit that dependant claims 2-11, 14-23, and 26-38 are likewise patentably distinguished over LeCluyse et al.[U] and Liu et al.[EE]. Applicant respectfully submits that the rejection of claims 1-11, 13-23, and 25-38 under 35 U.S.C.

Section 102(b), as being anticipated by LeCluyse et al.[U] or Liu et al.[EE], has been overcome, and withdrawal of this rejection is respectfully requested. Allowance of claims 1-11, 13-23, and 25-38 is also respectfully requested.

Response to the Rejection of Claims Under 35 U.S.C. Section 102(a)

Based on Liu et al.[CC]

Claims 1-64 are rejected under 35 U.S.C. Section 102(a) as being anticipated by Liu et al.[CC]. The Patent Office asserts states that Liu et al.[CC] disclose identical active steps and the identical structural elements of the subject invention as claimed. More specifically, the Patent Office states that Liu et al. [CC] disclose a method of screening various candidate compounds (parent compounds and metabolites thereof) including radiolabeled and fluorescent compounds, such as taurocholate, enkephalin, inulin, etc. for susceptibility to biliary excretion. Furthermore, the Patent Office states that Liu et al.[CC] also disclose the method of determining the *in vitro* biliary clearance value and teach that the resultant *in vitro* biliary clearance value corresponds to the *in vivo* biliary clearance.

Applicants respectfully submit the attached Declaration under 37 C.F.R. § 1.131 regarding Liu et al.[CC]. Summarily, the attached Declaration establishes that the intended subject matter of claims 1-64 was invented prior to the publication date of Liu et al.[CC]. Consequently, it is respectfully submitted that the rejection of claims 1-64 under 35 U.S.C. § 102(a) as being anticipated by Liu et al.[CC] has now been mooted. It is therefore respectfully requested that Liu et al.[CC] as a reference be withdrawn, and hence, that the rejections be withdrawn. Allowance of claims 1-64 is also respectfully requested.

Response to the Rejection of Claims Under 35 U.S.C. Section 103(a)

Based on LeCluyse et al.[U] and/or Liu et al.[EE] in view of

Liu et al.[EE], Poole et al.[V], and Dunn [A]

Claims 1-64 are rejected under 35 U.S.C. Section 103(a) as being unpatentable over LeCluyse et al.[U] and/or Liu et al.[EE] in view of Liu et al.[DD], Poole et al.[V], and Dunn [A]. In regard to Dunn [A] (U.S. Patent No. 5,602,026), the Patent Office asserts

that this particular reference teaches that sandwiched cultured hepatocytes have metabolic function of the liver *in vivo* and are therefore suitable for studying hepatocyte metabolism and recovering metabolite resulting from hepatocyte metabolism. The Patent Office further asserts that it would have been obvious to one skilled in the art to modify the methods of screening candidate compounds for susceptibility to biliary excretion of LeCluyse et al.[U] and/or Liu et al.[EE] by determining the values related to biliary excretion of candidate compounds as suggested by Liu et al.[DD] and Poole et al.[V]. The Patent Office also asserts that there would be a reasonable expectation of success in investigating susceptibility of candidate compounds to biliary excretion because hepatocytes maintained in sandwich cultures *in vitro* are known to have metabolic functions of the liver *in vivo* and that several various and specific parameters have been suggested in the prior art for evaluation and comparison of biliary clearance or biliary excretion of candidate compounds within *in vitro* and *in vivo* systems. This rejection is respectfully traversed.

Unlike the present invention, LeCluyse et al.[U] and Liu et al. [EE] do not teach, suggest, or motivate a method of providing an *in vitro* culture of hepatocytes having at least one bile canaliculus, which can be utilized for screening candidate compounds to indicate the susceptibility of a candidate compound to biliary excretion *in vivo*. By indicating the susceptibility of a candidate compound to biliary excretion *in vivo*, candidate compounds having an undesirably high susceptibility can optionally be eliminated early on from further evaluation as a potential therapeutic agent. Therefore, the subject invention enables early developmental drug screening to, in essence, "weed out" the therapeutic compounds that have only a minimal chance of imparting therapeutically beneficial effects upon a patient due to their high susceptibility to biliary excretion *in vivo*.

In marked contrast, LeCluyse et al. [U] are merely concerned with studying the effects on *in vitro* hepatocytes cultured in various collagen configurations in an effort to determine which collagen configuration would promote the formation of extensive *in vitro* bile canalicular networks so as to attempt to provide a more reliable representative model with which to study hepatic morphology and physiology. Given the abbreviated

nature of this abstract, Liu et al. [EE] merely disclose the measurement of *biliary excretion index* for selected model compounds in sandwich-cultured hepatocytes. Unlike the present invention, LeCluyse et al. [U] and Liu et al.[EE] do not teach, suggest, or motivate a method of screening compounds *in vitro* for determining the susceptibility of a compound to *in vivo* biliary excretion wherein an amount of candidate compound in the at least one bile canaliculi has been normalized. As a result, LeCluyse et al. [U] and Liu et al. [EE] suffer from a significant weakness in ability to predict *in vivo* excretion based on *in vitro* observations. Claims 1 and 13 have been amended to recite this aspect of the present invention, and are thus believed to be patentably distinguished over LeCluyse et al. [U] and Liu et al. [EE]. Support for this amendment can be found throughout the subject application as filed, including at page 30, lines 19-24. Normalizing the amount of candidate compound facilitates accurate extrapolation and scaling up of the *in vitro* observations to an *in vivo* setting, in accordance with the present invention.

Dunn [A] (U.S. Patent No. 5,602,026) does not support the above noted deficiencies in LeCluyse et al. [U] and in Liu et al. [EE]. Rather, the disclosure of Dunn is limited to broad generalizations stating that substances can be removed by the metabolic activity of the hepatocytes and that products of hepatocyte metabolism can be recovered from hepatocytes. This disclosure is not tantamount to teaching, or even motivating for that matter, a specific *in vitro* method of screening candidate compounds for determining the susceptibility of a candidate compound to *in vivo* biliary excretion.

Liu et al. [DD] merely discloses a comparison of the uptake of bile acid [^{3}H]-taurocholate (TC) in hepatocytes cultured in a sandwich configuration with that of hepatocytes cultured in a single layer collagen configuration in an effort to determine which system might be better suited for examining long-term activity and regulation of hepatobiliary transport systems. Given the abbreviated disclosure to this abstract, Liu et al.[DD] does not teach, suggest, or even motivate a method of screening a candidate compound, even a compound such as taurocholate, for susceptibility to biliary excretion in sandwich cultured hepatocytes.

Poole et al. [V] are concerned not with an *in vitro* method of *screening* candidate compounds for *susceptibility* to biliary excretion *in vivo*, but with investigating the cause of thyroid lesions that develop *in vivo* in rats following chronic treatment with temelastine. The Patent Office asserts that Poole et al.[V] teach a direct correlation of the *in vivo* biliary clearance of T_4 in rats with the *in vitro* accumulation of T_4 in cultured rat hepatocytes. However, Poole et al.[V], unlike the present invention, fails to teach, suggest, or even motivate a specific method of screening candidate compounds with cultured hepatocytes wherein an *in vitro* biliary clearance value is used as a tool for determining the susceptibility of a compound to *in vivo* biliary excretion within humans. In addition, Poole et al. [V] appears to teach away from the present invention, in that the method of the subject invention ultimately benefits human beings, whereas the studies conducted by Poole et al. [V] failed to exhibit any measurable effect in humans, suggesting that such a response to the presence of temelastine is species specific to rats.

Claim 25 recites a competition assay in accordance with the present invention, wherein a candidate compound and a marker compound are competed against each other to provide a method of screening a candidate compound for susceptibility to biliary excretion that is amenable to a high throughput screening approach. Thus, both a candidate compound and a marker compound are added to the culture for a time sufficient to allow for uptake so that the candidate compound and marker compound compete for uptake in the culture of hepatocytes. This aspect of the invention is now believed to be more particularly pointed out and distinctly claimed through the amendment of claim 25, step (d), to recite that the detecting of an amount of marker compound present in the at least one bile canaliculus in the culture is done "to evaluate uptake and excretion competition between the candidate compound and the marker compound." Support for this amendment can be found in the subject U.S. Patent Application as filed at page 22, line 8 through page 24, line 22, and particularly at page 22, lines 20-21. The evaluating of uptake and excretion competition between the candidate compound and the marker compound is not disclosed in the cited art of record, individually or in combination. Therefore, claim 25 and its dependent claims 26-

38 are believed to be patentably distinguished over the cited art of record. Allowance of these claims is respectfully requested.

Claims 39 and 52 have been amended to recite that the biliary clearance value is calculated by subtracting the normalized amount of the candidate compound present in the lysate of a second culture from an amount of candidate compound present in the lysate of a first culture and dividing by the time that the candidate compound was exposed to hepatocytes multiplied by the initial concentration of the candidate in the buffer medium. Support for this amendment can be found at page 19, lines 19-24 and at page 30, lines 19-24. There is no disclosure of the calculation of a biliary clearance value in the cited combination; much less any disclosure of the use of the calculation of a biliary clearance value as now presented in claims 39 and 52. Accordingly, claims 39 and 52 are believed to be patentably distinguished over the proposed combination of record, and allowance of claims 39 and 52 is respectfully requested.

Claim 52 has been further amended, at step (c), to recite the inducing of Phase I, Phase II or transport enzyme activity, or combinations thereof, in the hepatocytes of the first set of cultures. Support for this amendment can be found at page 21, lines 1-10, and at page 41, lines 12-24. This amendment is made to more particularly point out and distinctly claim this aspect of the subject invention in that Phase I and Phase II metabolic activities are typically associated with modification of a candidate compound by the liver *in vivo*. For example, such modification could occur wherein a pro drug is modified or converted to an active form. Specific embodiments of such activities are disclosed in Example 4 of the present U.S. Application as filed and include O-demethylation (such as by CYP3A4) and glucuronide conjugation. The induction of these metabolic activities is not disclosed in the proposed combination of references and accordingly, claim 52 is believed to be further patentably distinguished over the proposed combination.

Amended claims 1, 13, 25, 39, and 52 are believed to be distinguished from the cited combination. Claims 2-11 are dependent upon claim 1, claims 14-23 are dependent upon claim 13, claims 26-38 are dependent upon claim 25, claims 40-51 are dependent upon claim 39, and claims 53-64 are dependent upon claim 52. Therefore,

applicants respectfully submit that dependant claims 2-11, 14-23, 26-38, 40-51, and 53-64 are likewise patentably distinguished over the proposed combination of LeCluyse et al.[U] and/or Liu et al.[EE] in view of Liu et al.[DD], Poole et al. [V], and Dunn [A]. Therefore, applicants respectfully submit that the rejection of claims 1-11, 13-23 and 25-64 under 35 U.S.C. ' 103(a), as being unpatentable over LeCluyse et al. [U] and/or Liu et al. [EE] in view of Liu et al. [DD], Poole et al. [V], and Dunn [A], has been overcome. Withdrawal of this rejection is respectfully requested. Allowance of claims 1-11, 13-23, and 25-64 is also respectfully requested.

Discussion of New Claims

New claims 65 and 66 are dependent from claims 39 and 52, respectively. Each of these claims recite that the method of the present invention as set forth in claims 39 and 52 can further comprise the step of differentiating between candidate compounds that are not excreted in the bile, candidate compounds that are highly excreted in the bile, and candidate compounds that are rapidly and extensively excreted in the bile, using the biliary clearance value. Support for the addition of new claims 65 and 66 can be found at page 20, lines 1-8 of the subject U.S. Patent Application as filed, among other places. This aspect of the present invention is not believed to be disclosed in any of the prior art documents of record, and accordingly, claims 65 and 66 are believed to be patentably distinguished over the cited art of record. Allowance of claims 65 and 66 is therefore respectfully requested.

Supplemental Information Disclosure Statement

Applicants enclose herewith a Supplemental Information Disclosure Statement. Enclosed also herewith is a check covering the $180.00 fee associated with the submission of an Information Disclosure Statement after a first Office Action. It is respectfully requested that the enclosed Information Disclosure Statement and the references submitted with the Information Disclosure Statement be made of record in the prosecution of the present U.S. patent application.

CONCLUSION

In light of the above Amendments and Remarks, it is respectfully submitted that the present application is now in a proper condition for allowance and such action is earnestly solicited.

If any minor issues should remain outstanding after the Patent Examiner has had an opportunity to study the above Amendments and Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney so that all such matters may be resolved and the application be placed in a condition for allowance without the necessity for issuance of another Official Action.

The Commissioner is hereby authorized to charge any deficiencies of payment or credit any overpayments associated with the filing of this Amendment to Deposit Account No. 50-0426.

Respectfully submitted,

JENKINS & WILSON, P.A.

Date: September 13, 2001

By: _____
Arles A. Taylor, Jr.
Registration No. 39,395

Suite 1400 University Tower
3100 Tower Boulevard
Durham, North Carolina 27707
Telephone: (919) 493-8000
Facsimile: (919) 419-0383

Customer No. Bar Code Label:

25297
PATENT TRADEMARK OFFICE

421/17/2 AAT/rel

Enclosures: Postcard
Petition
Declaration
Check in the amount of $625.00
Supplemental Information Disclosure Statement

Version With Markings To Show Changes Made

IN THE CLAIMS:

Please cancel claims 12 and 24.

Claims 1, 13, 25, 39, and 52 have been amended as follows:

1. (Once Amended) A method of screening a candidate compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

(a) providing a culture of hepatocytes, the culture of hepatocytes comprising at least one bile canaliculus;

(b) exposing a candidate compound to the culture; and

(c) determining [an] a normalized amount of candidate compound in the at least one bile canaliculus, the amount of the candidate compound in the at least one bile canaliculus indicating the susceptibility of the candidate compound to biliary excretion.

13. (Once Amended) A method of screening a plurality of candidate compounds simultaneously *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising:

(a) providing a plurality of cultures of hepatocytes, wherein each culture of hepatocytes comprises at least one bile canaliculus;

(b) exposing a different candidate compound within the plurality of candidate compounds to each culture within the plurality of cultures; and

(c) determining [an] a normalized amount of candidate compound in the at least one bile canaliculus, the amount of the candidate compound in the at least one bile canaliculus indicating the susceptibility of the candidate compound to biliary excretion.

25. (Once Amended) A method of screening a candidate compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

(a) providing a culture of hepatocytes, the culture comprising at least one bile canaliculus;

(b) exposing a candidate compound and a pre-selected amount of a marker compound to the culture for a time sufficient to allow uptake;

(c) washing the culture; and

(d) detecting an amount of marker compound present in the at least one bile canaliculus in the culture to evaluate uptake and excretion competition between the candidate compound and the marker compound, the presence or the absence of a reduced amount of the marker compound as compared to the pre-selected amount of marker compound indicating the susceptibility of the candidate compound to biliary excretion.

39. (Once Amended) A method of screening a candidate compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

(a) establishing first and second cultures of hepatocytes, each culture comprising at least one bile canaliculus, the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi;

(b) exposing a candidate compound to the first culture and to the second culture for a time sufficient to allow uptake of the candidate compound;

(c) washing and then lysing the first and second cultures;

(d) [measuring an] determining a normalized amount of candidate compound present in a lysate obtained from each culture in step (c); and

(e) calculating a biliary clearance value [derived from the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi using the amount of candidate compound in each culture lysate measured in step (d)] by subtracting: (the normalized amount of the candidate compound present in the lysate of the second culture, as measured in step (d), from the normalized amount of the candidate compound present

in the lysate of the first culture, as measured in step (d)) and dividing by (the time of step (b) that the candidate compound was exposed to the hepatocytes multiplied by an initial concentration of the candidate compound in a buffer medium), the calculated biliary clearance value indicating the susceptibility of the candidate compound to biliary excretion.

52. (Once Amended) A method of screening a metabolite of a candidate parent compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

(a) establishing a first set and second set of two cultures of hepatocytes, each culture comprising at least one bile canaliculus, a first culture within each set having intact bile canaliculi and a second culture within each set having disrupted bile canaliculi;

(b) exposing a candidate parent compound to the first culture and to the second culture of each set for a time sufficient to allow uptake of the candidate parent compound;

(c) inducing Phase I, Phase II, or transport metabolic enzyme activity, or combinations thereof, in the hepatocytes of the first set of cultures;

(d) washing and lysing the first and second cultures of each set;

(e) [measuring an] determining a normalized amount of candidate parent compound present in a lysate obtained from each culture in step (d);

(f) [measuring an] determining a normalized amount of the metabolite of the candidate parent compound present in a lysate obtained from each culture in step (d);

(g) calculating a biliary clearance value [derived from the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi using the amount of candidate parent compound in each culture lysate measured in step (e)] for the candidate parent compound by subtracting (the normalized amount of the candidate parent compound present in the lysate of the second culture, as measured in step (e), from the normalized

amount of the candidate parent compound present in the lysate of the first culture, as measured in step (e)) and dividing by (the time of step (b) that the candidate parent compound was exposed to the hepatocytes multiplied by an initial concentration of the candidate compound in a buffer medium), the calculated biliary clearance value indicating the susceptibility of the candidate parent compound to biliary excretion; and

(h) calculating a biliary clearance value [derived from the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi using the amount of the metabolite of the candidate parent compound in each culture lysate measured in step (f)] for the metabolite of the candidate parent compound by subtracting (the normalized amount of the metabolite of the candidate parent compound present in the lysate of the second culture, as measured in step (f), from the normalized amount of the metabolite of the candidate parent compound present in the lysate of the first culture, as measured in step (f)) and dividing by (the time of step (b) that the candidate parent compound was exposed to the hepatocytes multiplied by an initial concentration of the candidate compound in a buffer medium), the calculated biliary clearance value indicating the susceptibility of the metabolite of the candidate parent compound to biliary excretion.

Please add the following new claims:

65. (New) The method of claim 39, further comprising the step of differentiating between a candidate compound that is not excreted in bile, a candidate compound that is highly excreted in bile, and a candidate compound that is readily and extensively excreted in bile, using the biliary clearance value.

66. (New) The method of claim 52, further comprising differentiating between a candidate compound that is not excreted in bile, a candidate compound that is highly excreted in bile, and a candidate compound that is rapidly and extensively excreted in bile, using the biliary clearance value.